

EXHIBIT A

File No.: 16408-3US

Ottawa, Canada
December 14, 2004



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Andrew C. KARAPLIS

For : USE OF PEX IN THE TREATMENT OF METABOLIC BONE DISEASES

Serial No. : 09/806,110

Filed : 08/31/2001

Examiner : Ram R. SHUKLA

Art Unit : 1632

DECLARATION Under 37 CFR 1.132

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
U. S. A.

Sir:

I, Arthur E. Broadus, M.D., Ph.D., am a citizen of the United States of America and hold a Doctor of Medicine degree and a Ph.D. in Physiology. I hold the position of Ensign Professor of Medicine and Section Chief, Endocrine Division, at the Yale University School of Medicine, New Haven, Connecticut, U.S.A.

I have more than 28 years experience in the field of medicine and, MAKE OATH AND SAY:

1. THAT, I have reviewed and understand the subject matter of the above-captioned U.S. application.

2. THAT the purpose of this Declaration is to convey my understanding and knowledge of the subject matter of the above-captioned U.S. Patent Application, and supporting evidence as herein provided as Exhibit B; and to provide my opinion thereof in relation to support for the claims of US Patent application S.N. 09/806,110 and in view of the teachings of Vickery in US Patent 6,583,114 and Chorev et al. in WO 96/19246, as currently cited by the Examiner against the subject matter of claims 7-10 of the present application.

3. THAT, in view of the rising incidence of bone diseases, such as osteoporosis, especially in the increasingly elderly population, there is a corresponding demand for effective methods of treating such metabolic bone diseases.

4. THAT it is known in the art that PTH and PTHrP are anabolic agents for bone and are the only two truly anabolic agents described to date; other agents in use may decrease bone loss and thereby have therapeutic potential, but only PTH and PTHrP cause an actual therapeutic increase in bone formation. This anabolic response is seen with intermittent PTH or PTHrP administration, as when they are administered daily.

5. THAT the above-captioned application (supplemented by Appendix B) shows that PEX (now "PHEX") is a type II integral membrane endopeptidase that is capable of cleaving PTH and PTHrP. This capacity to cleave PTH and PTHrP is unique to PEX and is not seen in other members (NEP or ECE) of the same endopeptidase family. PEX is highly expressed in bone and on osteoblastic bone cells.

6. THAT based on the findings of the above-captioned application, PTH and PTHrP are substrates of PEX. This point was established *in vitro* by using PEX-transfected cells that were capable of carrying out PTH cleavage and by using osteoblastic UMR cells to demonstrate cleavage of PTHrP, the latter experiment documenting that endogenous or native PTHrP is a PEX substrate.

7. THAT based on the findings of the above-captioned application, I would clearly understand that inhibition of PEX would be expected to result in 1) an increase in local endogenous PTHrP levels in or bound to osteoblasts and 2) would also be expected to prolong the "on time" of PTH bound to local osteoblastic PTH/PTHrP receptors and thereby potentiate PTH effects on these cells.

8. THAT I believe the above-noted application provides factual evidence and soundly predicts that PEX inhibitors, such as phosphoramidon, would result in an increase in PTH and/or PTHrP levels in the microenvironment of PTH/PTHrP receptors on osteoblasts, which would in turn lead to increase in bone formation. This approach represents a novel method of regulating bone formation that could form the basis of a novel therapy for osteopenic/osteoporotic states in humans. Indeed, this approach is sufficiently novel that a subsequent application from another party that described a compound that had such actions would be anticipated to form the basis of a successful use patent.

9. THAT the inventors completed an *in vitro* study using UMR-106 osteoblast cultured cells that were examined with respect to PTH/PTHrP mRNA levels (using RT-PCR) and PTH/PTHrP protein levels (using an immunoradiometric assay) in the presence of PBS/Pho or PBS (control); and wherein the finding of this study is summarized in Figure 1 of EXHIBIT B herein provided.

10. THAT the above *in vitro* study confirms that the Pho, an inhibitor of PEX, resulted in an increase in PTHrP protein levels in the osteoblast microenvironment. Based on what is known with respect to PTHrP and bone formation, an increase in PTHrP levels would be expected to increase bone formation.

11. THAT the above *in vitro* study supports the findings of the above-noted application, namely that PTH/PTHrP are substrates of PEX, and the inhibition of PEX activity results in an increase in osteoblast PTHrP levels.

12. THAT the above *in vitro* study supports that Pho inhibits PEX, where in the presence of Pho there is an increase in PTHrP levels, which further confirms that PTHrP is a substrate of PEX.

13. THAT the findings of the above *in vitro* study were further supported by an *in vivo* study to determine if the inhibition of PEX in fact results in an increase in bone formation, based on the maintenance of a serum bone marker indicative of bone formation.

14. THAT osteocalcin levels are known in the art to be directly proportional to bone formation levels; wherein an increase in osteocalcin level indicates an increase in bone formation.

15. THAT the inventors completed an *in vivo* study using eight C57BL/6 mice, where mice into 2 groups of 4: where (a) two mice injected daily for 14 days with PBS (control mice) and (b) two mice injected daily for 14 days with PBC/Pho; while (c) two mice injected daily for 36 days with PBS (control mice) and (d) two mice injected daily for 36 days with PBC/Pho; and the osteocalcin levels were measured at day 14 and day 36. The findings of this *in vivo* study are summarized in Figure 2 of EXHIBIT B herein provided.

16. THAT the above *in vivo* results show that osteocalcin levels were maintained in Pho-treated mice at day 36, whereas they were decreased in the PBS-treated control mice at day 36. The decrease in circulating osteocalcin in the control mice at day 36 is the expected result based on the progressive decrease in growth and bone formation in mice as they age, so that maintaining an elevated level of osteocalcin in the Pho-treated mice represents evidence that a high rate of bone formation continued in these mice.

17. THAT serum bone markers such as osteocalcin have been used in the clinical setting as predictors of a response to therapeutic treatment and have been used as surrogates for therapeutic responses before observed changes are or can be recorded by bone density measurements (or other techniques used to monitor bone mass).

18. THAT the *in vivo* results provide strong confirmatory evidence that inhibition of PEX may provide a novel method of treating osteopenic/osteoporotic bone disease.

19. THAT the results *in vitro* and *in vivo* combined suggest that activation of PEX would result in a decrease in local osteoblastic PTH/PTHrP levels, with a consequential decrease in bone formation. In this circumstance, osteocalcin levels would be expected to be decreased. This approach would represent a novel strategy for approaching osteosclerotic bone disorders in humans.

20. THAT PTH and PTHrP are powerful bone-forming agents and, as noted above, are the only two such bone anabolic agents known at present. Nevertheless, these two agents are biologically distinct. PTH is a systemic peptide hormone that acts on bone if and when its secretion is stimulated systemically by a reduction in the serum calcium concentration. In contract, PTHrP does not normally circulate but rather is an endogenous autocrine/paracrine product of osteoblasts resident in bone; germ-line haploinsufficiency of PTHrP, as in the heterozygous PTHrP-null mouse (Amizuka N, et al., Dev. Biol., 1996, 175, 166-176), or conditional deletion of PTHrP in osteoblasts (Miao D, et al., J. Bone Min. Res., 2002, 17 (Suppl), S138), both result in quite striking osteopenia/osteoporosis, demonstrating that PTHrP is a locally-produced anabolic factor in bone.

Thus, local inhibition of PEX in osteoblasts would be expected to increase the effects of both circulating PTH and local PTHrP, and this mechanism is clearly unique to the novel approach described in the present application. The anabolic potential of this approach as it regards the anabolic effects of local PTHrP is abundantly clear from the heterozygous PTHrP-null mouse and the conditional knockout mouse findings noted above.

21. THAT based on my expertise in this area, the findings described in the above noted application and Appendix B indicate that the inhibition of PEX constitutes an entirely novel approach toward modulating the effects of systemic PTH and local osteoblastic PTHrP, which effects, singly or in combination, would be expected to be anabolic in bone.

22. THAT, I understand the teachings of Vickery et al to involve the systemic use of PTHrP analogs to enhance fracture healing in healing and/or non-healing fractures of long bones. In so far as my reading of the patent accurately describes its claims, these are limited to fracture healing and to systemic administration of PTHrP analogs. These claims do not include anabolic effects in bone per se nor do they involve a technique for modulating the effects of endogenous PTH or PTHrP in bone.

23. THAT I understand the teachings of Chorev et al to include the continuous low-dose administration of PTH, PTHrP or related compounds by systemic administration as a means of promoting bone formation. Key words here are "continuous" and "low-dose", for higher doses of continuously administered such peptides/analogues would be anticipated to result in catabolic rather than anabolic effects in bone. One can only presume that patents exist whereby PTH and related compounds are administered intermittently as anabolic agents and that these patents are the basis of the commercial anabolic PTH products presently marketed in the United States. One further assumes that patent of Chorev et al differs from such patents in the analogues claimed and/or in its use of continuous, low-dose products. In any event, the Chorev et al patent involves systemic administration of PTH/PTHrP and analogues and does target an anabolic effect in bone. However, here too, the approach differs entirely from the strategy involved in the above-captioned application, in that what Chorev et al teaches is neither targeted at PEX nor designed to modulate the effects of endogenous circulating PTH or local osteoblast-derived PTHrP. It merits emphasis here that

modulating PEX activity and therefore the presumed effects of circulating PTH and/or local osteoblastic PTHrP would, in essence, magnify the effects of endogenous agents that had been called into play by physiological stimuli either at the level of the parathyroids and/or at the level of the osteoblast. In either case, or both, magnifying such effects might be seen as physiologically based or, at least, more physiologically based than simply administering pharmacological products systemically.

24. THAT, I believe the findings of the above-noted application and the results as herein provided distinguish this application and its strategy very clearly from the teachings of either Vickery et al or Chorev et al. Specifically, and as noted in several preceding sections, the above-noted application has as its strategy the modulation of PEX activity, which would in turn magnify the effects of endogenous circulating PTH and/or the effects local osteoblast-derived PTHrP in bone. The entire strategy here differs from the simple systemic administration of PTH/PTHrP or any analog thereof and is therefore entirely novel.

25. THAT I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.


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Declaration of Arthur E. Broadus
December 14, 2004

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FURTHER AFFIANT SAYETH NOT.

SIGNED AT New Haven, in the State of Connecticut, this 14th day of Dec. 2004.

SWORN before me at the City)
of New Haven, in the)
Province of Connecticut,)
this 14th day of Dec. 2004.)



Arthur E. Broadus

Bailara J. Wauciak-Dillon 11/30/2005
Notary Public exp.

CURRICULUM VITAE

Name: Arthur Eastwood Broadus
Born: March 28, 1941 -- Knoxville, Tennessee
Marital Status: Married (Carole Kepler Dickinson)
Children: Courtney Eastwood Broadus
Elizabeth Dickinson Broadus

Education:

1. The Lawrenceville School, Lawrenceville, NJ, 1956-60. Harvard Book Award.
2. B.A., Washington and Lee University, Lexington, VA, 1960-64. Gilliam Award.
3. M.D., Vanderbilt University School of Medicine, Nashville, TN, 1964-66 and 1969-71. Founder's Medal (first standing in class), Borden Award, Roche Award, Alpha Omega Alpha.
4. Ph.D., Vanderbilt University, Department of Physiology, 1966-69; thesis advisor, Earl W. Sutherland, Jr.

Positions Held:

1. Intern in Medicine, Massachusetts General Hospital, Boston, MA, 1971-72.
2. Assistant Resident in Medicine, Massachusetts General Hospital, Boston, MA, 1972-73.
3. Staff Associate, Endocrinology Branch, National Heart and Lung Institute and Endocrine Fellow, Combined Endocrine Training Program, National Institutes of Health, Bethesda, MD, 1973-76.
4. Assistant Professor of Internal Medicine, Yale University, New Haven, CT, 1976-80.
5. Associate Professor of Internal Medicine, term appointment, Yale University, New Haven, CT, 1980-84.
6. John Simon Guggenheim Fellow and Visiting Associate Professor of Medicine, Harvard Medical School, Boston, MA, 1982-83.
7. Associate Professor of Internal Medicine, without term, Yale University, New Haven, CT, 1984-87.
8. Section Chief, Division of Endocrinology and Metabolism, Yale University, 1986-present.
9. Professor of Internal Medicine, Yale University, New Haven, CT, 1987-present.
10. Professor of Cellular and Molecular Physiology, Yale University, New Haven, CT, 1990-present.
11. Ensign Professor of Internal Medicine, Yale University, New Haven, CT, 1994-present
12. Associate Chair for Research Programs, Department of Internal Medicine, Yale University, New Haven, CT, 1994-present

Society Membership and Honors:

Alpha Omega Alpha, American Federation of Clinical Research, Endocrine Society, American Society for Bone and Mineral Research, American Society for Clinical Investigation, John Simon Guggenheim Fellow (1982), Fellow of Davenport College, Yale University (1986-), Frederic C. Bartter Award of the American Society for Bone and Mineral Research (1989), American Association of Physicians (1990-), Council of the American Society for Bone and Mineral Research (1990-93), NIH Merit Award (1992-), Program Chair, Second combined meeting of the American Society for Bone and Mineral Research and the International Bone and Mineral Society (1998).

Military Service: United States Public Health Service, 1973-76.

Certification: American Board of Internal Medicine, 1974.

Study Sections:

1. Career Development Study Section, Veterans Administration, 1984-87.
2. Scientific Advisory Committee for Biochemistry and Endocrinology, American Cancer Society, 1990-94 (Chair 1993-94).
3. Howard Hughes Medical Institute Medical Student Fellowship Committee, 1992-94.
4. NIH General Medicine B Study Section, 2000-present.

Editorial Boards:

1. Journal of Clinical Endocrinology and Metabolism, 1982-87.
2. American Journal of Physiology, 1988-.
3. Journal of Clinical Investigation, 1989-94.
4. Bone, 1990-.
5. Bone and Mineral, 1991-.

Publications: Listing attached.

PUBLICATIONS**Peer-reviewed Articles**

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9. Broadus AE, LJ Deftos, FC Bartter. Effects of the intravenous administration of calcium on nephrogenous cyclic AMP: evaluation as a parathyroid suppression test. *J Clin Endocrinol Metab* 46:477, 1978.
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11. Broadus AE, M Dominguez, FC Bartter. Pathophysiologic studies in idiopathic hypercalciuria. *J Clin Endocrinol Metab* 47:751, 1978.
12. Broadus AE. Nephrogenous cyclic AMP as a parathyroid function test. *Nephron* 23:137, 1979.
13. Broadus AE, SO Thier. Metabolic basis of renal stone disease. *N Engl J Med* 300:839, 1979.
14. Brown EM, AE Broadus, MF Brennan, GD Aurbach, SJ Marx, AM Spiegel, RW Downs, M Attie, GD Aurbach. Direct comparison *in vivo* and *in vitro* of suppressibility of parathyroid function by calcium in primary hyperparathyroidism. *J Clin Endocrinol Metab* 48:604, 1979.

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16. Gertner JM, R Horst, AE Broadus, H Rasmussen, M Genel. The effects of human growth hormone replacement on parathyroid function and vitamin D metabolism. J Clin Endocrinol Metab 49:185, 1979.
17. Mahnensmith R, SO Thier, RC Cooke, A Broadus, RA DeFronzo. The effect of acute metabolic acidemia on renal electrolyte transport in man. Metabolism 28:831, 1979.
18. Broadus AE, RL Horst, R Lang, ET Littledike, H Rasmussen. The importance of circulating 1,25-dihydroxyvitamin D in the pathogenesis of hypercalciuria and renal stone formation in primary hyperparathyroidism. N Engl J Med 302:422, 1980.
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22. Goltzman D, AF Stewart, AE Broadus. Malignancy-associated hypercalcemia: evaluation with a cytochemical bioassay for parathyroid hormone. J Clin Endocrinol Metab 53:899, 1981.
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25. Stewart AF, R Romero, PE Schwartz, EI Kohorn, AE Broadus. Hypercalcemia associated with gynecologic malignancies. Biochemical Characterization. Cancer 49:2389, 1982.
26. Insogna KL, AE Broadus, JM Gertner. Impaired phosphorus conservation and 1,25-dihydroxyvitamin D generation during phosphorous deprivation in familial hypophosphatemic rickets. J Clin Invest 71:1562, 1983.
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